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The role of fluorescence in situ hybridization for predicting recurrence after adjuvant Bacillus Calmette-Guérin in intermediate- and high-risk non-muscle invasive bladder cancer patients: a systematic review and meta-analysis of individual patient data

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Abstract

Purpose

The objective of this study was to assess the value of fluorescence in situ hybridization (FISH) for predicting early recurrence in intermediate-, and high-risk NMIBC patients treated with BCG.

Materials and methods

A systematic review was conducted using MEDLINE, Embase and the Cochrane library. Individual patient data (IPD) from prospective observational studies evaluating FISH in patients treated with BCG were included. A two-stage IPD meta-analysis was carried out to assess the value of FISH for predicting tumor recurrence after BCG induction therapy.

Results

From four studies IPD were obtained of 422 patients, and 408 patients included in final analysis with a median follow-up of 18.8 months. The hazard ratio for recurrence when FISH was positive was pre-BCG (t_0) 1.20 (95% CI: 0.81–1.79), at six weeks (t_1) 2.23 (95% CI: 1.31–3.62), at three months (t_2) 3.70 (95% CI: 2.34 – 5.83), and at six months (t_3) 23.44 (95% CI: 5.26–104.49).

Conclusion

A positive FISH test post-BCG correlates with a higher risk for a tumor recurrence. FISH could aid urologists in risk stratification and counseling of patients. Based on both HR and its narrowest CI, the preferred timing for FISH is three months following TURBT. This is also in time for patients who fail to respond to induction therapy to enter clinical trials, or to change treatment strategy.

Key words

Urinary bladder neoplasms; mycobacterium bovis; in situ hybridization, fluorescence; recurrence; meta-analysis

Introduction

Intravesical Bacillus Calmette-Guérin (BCG) is recommended by international guidelines for treatment of intermediate-, and high-risk non-muscle invasive bladder cancer (NMIBC).[1–4] However, up to 40% of patients develop a recurrence despite BCG therapy and are exposed to the risk of progression as well as to its local and systemic side effects.[5–9] Early identification of recurrence could minimize these risks and other treatment options can be considered at an earlier stage.

Currently, follow-up of high-risk tumors is recommended with cystoscopy and urinary cytology. Cytology has a high sensitivity for grade 3 (high-grade) tumors, but a low sensitivity in grade 1 (low-grade) tumors. However, BCG treatment can hamper cytological evaluation, and is therefore less reliable after BCG therapy.[10,11] The UroVysion® fluorescence in situ hybridization (FISH) test detects chromosomal aberrations, associated with bladder cancer, and is not influenced by the BCG-induced inflammatory response.[12,13] In patients receiving BCG, several small studies have described a positive role of FISH in predicting recurrence following BCG instillations. To obtain more convincing evidence, we performed a systematic review and meta-analysis of individual patient data (IPD) from available studies assessing the prognostic value of FISH following BCG instillations for NMIBC.

Materials and methods

Protocol and registration

This systematic review and IPD meta-analysis was registered in the PROSPERO international prospective register of systematic reviews (registration number CRD42018077631), and is reported following the Preferred Reporting Items for Systematic Reviews and Meta-analysis of Individual Participant Data statement.[14] Ethical approval was documented in the original publications of all studies.

Eligibility criteria and literature search

All prospective observational studies that evaluated FISH for tumor recurrence in NMIBC patients treated with BCG therapy (induction with or without maintenance) were eligible. A systematic literature search was conducted using MEDLINE (via PubMed), Embase and the Cochrane library (including the Cochrane Database of Systematic reviews and the Cochrane Central Register of Controlled Trials), without restrictions. The search strategy, outlined in *Supplemental table 1*, was conducted on September 7, 2017 and updated on September 6, 2018. The reference lists of the included studies were examined for additional studies.

Study selection and risk of bias

Two independent investigators (EL, RV) screened all identified titles and abstracts. Full-text papers of all candidate studies were retrieved. These studies were reviewed (EL, RV) and disagreements about study inclusion were resolved by a third investigator (TdR). Risk of bias was assessed according to the Quality In Prognosis Studies tool (EL, RV).[15]

IPD collection and data integrity

IPD for all eligible clinical trials were requested on (1) baseline characteristics including patients demographics and clinico-pathological characteristics; (2) timing of FISH tests and their results; and (3) clinical outcome, including time to recurrence and histopathology of recurrence. Before pooling the data into a single database, the data of all included trials were carefully checked. Any discrepancies were discussed and resolved with the authors.

Specification of outcomes and effect measures

The value of FISH at different time points was evaluated for predicting tumor recurrence in patients treated with BCG. Recurrence was defined as a histologically proven bladder tumor. Its predictive value for progression to muscle invasive disease (stage ≥T2) was a secondary outcome. FISH tests were considered positive according to the definition in the individual studies. In assessment of FISH tests, some studies considered tetraploid cells as normal and some studies considered them as aberrant cells. For this study, tetraploid cells were considered aberrant cells. In case the original study reported a negative FISH test despite the presence of tetraploid cells, the FISH test was considered positive in the current study when the definition of a positive FISH test was met due to the tetraploid cells.

Synthesis methods

IPD at baseline and during follow-up were collected from all participating studies. Patients lacking all cystoscopic follow-up data or missing all FISH evaluations were considered incomplete and were excluded from the analyses. Patient-, and disease-specific characteristics were explored across studies using descriptive statistics. Follow-up time was calculated as time since initial transurethral resection of the bladder tumor (TURBT) to date of histologically proven recurrence, or last follow-up.

Fixed-effect two-stage IPD meta-analysis forest plots were calculated. Heterogeneity across studies was assessed with the Cochrane Q chi-squared test and Higgins I^2. We assumed no clinical heterogeneity between studies concerning population, intervention and outcome. Therefore, we conducted fixed effect meta-analyses. Hazard ratios, including their respective 95% confidence intervals (95% CI) were calculated with Cox regression analysis. Positive predictive value (PPV) and negative predictive value (NPV) were calculated using 2x2 tables. For the time-to-event outcomes, including time to recurrence and time to disease progression, the starting point was the date of the initial TURBT. The two time-to-event outcomes were estimated by Kaplan-Meier analysis with recurrence or disease progression as the event. Patients who died of other causes prior to recurrence or progression were censored. The time-to-event distributions were compared using the log-rank test. For both the meta-analysis forest plots and Kaplan Meier analysis, a Landmark analysis was also performed for which patients with a recurrence at or before the landmark were excluded. The different points in time when FISH was performed were considered as landmark. Exploratory subgroup analyses were conducted based on a-priori defined subgroups. All tests were two-sided using 0.05 as the significance level. All analyses were performed using Stata/MP version 15.1.

Results

Study selection and availability of IPD

The systematic search (*Figure 1*), identified six eligible studies.[16–21] For two studies, the principal investigators no longer had access to IPD.[16,18] IPD were finally available for four cohort studies, resulting in a total of 422 patients.[17,19–21]

Risk of bias within studies

In general, risk of bias of all four studies was comparable, with low risks of bias except for study attrition and confounding. The risk of bias assessment is listed in *Table 1*.

Study and patient characteristics

The main characteristics of the included studies are summarized in *Table 2*. Fourteen patients were excluded from all analyses (1 missing follow-up data, 13 missing all FISH results), resulting in a total of 408 patients included in the final analysis. In *Table 3* baseline and tumor characteristics are summarized. Median follow-up was 18.8 months (interquartile range [IQR] 10.2–28.0 months).

Pooled analyses regarding recurrence

Out of 408 patients, 141 patients (34.6%) developed a recurrence during follow-up. Median time from initial TURBT to recurrence was nine months (IQR 5–16 months). For five patients a tumor recurrence was reported in the original study, but only based on high-grade (grade 3) cytology. In the current analysis these five patients were not scored as having a histologically proven tumor recurrence. Two studies considered tetraploid cells as normal and two studies considered tetraploid cells as aberrant cells. Nineteen negative FISH tests reported in the original studies showed tetraploid cells. For this analysis the tetraploid cells were considered aberrant, and the FISH test was considered positive when the definition of a positive FISH test was met (*Table 2*). Subsequently, 13 of these 19 FISH tests met the definition of a positive FISH test and were considered positive.

FISH results were collected at four different time points (t_0: pre-BCG; t_1: at the end of BCG induction at six weeks; t_2: at three months after initial TURBT; t_3: at six months after initial TURBT), although not all studies provided data at each of these time points (*Supplemental Figure 1*). FISH results and occurred conversions are displayed in *Table 4* and *5*. Evaluation of bladder recurrences was performed by cystoscopy followed by histological confirmation.

Predictive value of FISH for recurrence

The predictive value of FISH was determined for the different time points (t_0, t_1, t_2 and t_3). For t_1, t_2, and t_3, landmark analyses were performed.

At t_0, FISH results were available for 374 patients. A recurrence occurred in 133 patients (35.6%; 43 FISH negative [30.3%], 90 FISH positive [38.8%]). A positive FISH at t_0 was not associated with a higher risk for recurrence (HR 1.20, 95% CI: 0.81–1.79) (*Figure 2A*). Fixed-effect meta-analysis showed no heterogeneity (I^2=28.0%, p=0.244). PPV was 67.7%, and NPV was 69.7%.

At t_1, 249 FISH evaluations were available. In 84 patients (33.7%) a tumor recurrence occurred during follow-up (44 FISH negative [26.2%], 40 FISH positive [49.4%]). A positive FISH at t_1 was associated with a higher risk for recurrence (HR 2.23, 95% CI: 1.37–3.62) (*Figure 2B*). Meta-analysis showed moderate heterogeneity (I^2=51.3%, p=0.152). PPV and NPV were respectively 47.6% and 73.8%.

At t_2, 303 FISH evaluations from all studies were available. In 103 patients (34.0%) a recurrence developed during further follow-up (35 FISH negative [18.8%], 68 FISH positive [58.1%]). A positive FISH at t_2 was associated with a higher risk for recurrence (HR 3.70, 95% CI: 2.34–5.83) (*Figure 2C*). Meta-analysis showed no heterogeneity (I^2=0.0%, p=0.676). PPV and NPV were 66.0% and 81.2%, respectively.

At t_3, 71 patients with FISH evaluations were available from one trial and in 19 patients (26.8%) a recurrence occurred during further follow-up (4 FISH negative [8.2%], 15 FISH positive [68.2%]). A positive FISH at t_3 was associated with a higher risk for developing a recurrence (HR 23.44, 95% CI: 5.26–104.49) (*Figure 2D*), though this should be interpreted with caution given the wide 95% CI. PPV was 78.9% and NPV was 91.8%.

Analysis for recurrence-free survival

Kaplan-Meier curves for the different time points are shown in *Figure 3*. For t_1, t_2, and t_3 landmark analyses were performed. Log-rank test did not show an association between a positive FISH test pre-BCG (t_0) and tumor recurrence (p=0.160). However, a positive FISH test following BCG induction therapy (t_1, t_2, or t_3) was associated with a higher risk of tumor recurrence (all p<0.005).

Predictive value of FISH for progression to muscle-invasive disease (T ≥2)

Disease progression to muscle-invasive bladder cancer occurred in 17 patients (4.2%). At t_0, 374 patients had available results, of which 15 patients (4.0%) showed progression (3 FISH negative, 12 FISH positive). Forest plots at different time points are provided in *Supplemental figure 2.* Due to insufficient numbers, no reliable conclusions could be drawn from this.

Subgroup analyses

Subgroup analyses were performed for t_0 and t_2. At these time points all studies performed FISH tests. The following subgroups were predefined: age, gender, recurrent versus primary disease, presence of carcinoma in situ (CIS), prior BCG treatment, and BCG maintenance therapy versus BCG induction only. Patients with a positive FISH at t_0 without CIS had a statistically significant ($p=0.043$) higher risk for developing recurrence compared to patients with CIS and a positive FISH (HR 1.32, 95% CI: 0.91–1.91). No significant differences were found within the other subgroups, at either t_0 or t_2 (*Figure 4*).

Discussion

This IPD meta-analysis is the largest study evaluating FISH for predicting tumor recurrence and confirms that patients with a positive FISH test following BCG induction therapy have a higher risk for developing tumor recurrence during follow-up. The pooled analysis at t_2 showed a HR of 3.70. This confirms the conclusion of all four included studies. Two included studies that evaluated the predictive value of FISH at t_1, i.e. the end of the BCG induction therapy, reported inconsistent results, possibly due to low number of patients.[20,21] Within the current pooled analysis, a positive FISH test at 6 weeks is associated with a higher risk of recurrence during follow-up (HR 2.23). One of the included studies,[20] as well as one of the studies for which IPD could not be obtained,[16] reported that FISH had a predictive value for recurrence, when performed before BCG therapy was started (t_0). However, this effect was not seen in the pooled analysis. A recently published study of Lotan et al. reported similar results for t_1 and t_2, but also reported a positive association at t_0, in contrary to the current pooled analysis. [22] The HR of 23.44 at t_3 seems high and promising, however the wide 95% CI (5.26–104.49) makes this result unreliable. The results regarding FISH for predicting progression to muscle invasive disease should be interpreted with caution because of the limited number of events.

Subgroup analyses revealed a significant difference at t_0 for presence of CIS. In patients without CIS, a positive FISH pre-BCG was associated with a higher risk of recurrence. For this group of patients, a positive FISH following TURBT might be suggestive for residual tumor. However, at t_2 this difference was not seen anymore. A positive FISH at t_2 may be related to an insufficient response to BCG.

An overall false positive rate of FISH after BCG induction therapy of 41% seems high (*Table 4*). However, it is possible that a median follow-up 1.5 years was too short to identify all future recurrences. Most recurrences occur within 5 years of initial BCG induction therapy, though recurrence after 10 years are not unusual.[23,24]

Kamat et al. developed a CyPRIT-nomogram to predict BCG response based on changes in levels of a combination of nine cytokines in urine samples.[25] Both the FISH test and CyPRIT-nomogram identify a new group of patients, who show a molecular or cytokine failure, without clinical tumor present yet. This may assist in risk stratification and introduces the opportunity to offer these patients other subsequent treatment strategies at an earlier stage that hopefully will result in a better outcome. Treating all patients with a positive FISH test with a radical cystectomy may be too rigorous, but discussing clinical trials or changing BCG maintenance therapy to e.g. chemohyperthermia could be a viable option.[26,27] When FISH is performed in patients treated with BCG, we recommend to perform FISH at 3 months, since this has the highest HR with the narrowest 95% CI.

A limitiation of this IPD meta-analysis is that for two studies no IPD could be obtained (79 patients treated with BCG, mitomycin C or thiothepa). Also, the IPD of Lotan et al. could not be included in the current study since their publication is so recent, though all studies reported a higher risk for tumor recurrence in case of a positive FISH after BCG induction therapy. Another limitation of this analysis is that one study had a slightly different definition of a positive FISH test (*Table 2*). Though it is not likely that this would change the results of the present analysis, the more compliant

FISH criteria could have led to an overestimation of positive FISH tests. There was no uniform BCG maintenance protocol across the studies, which could also have influenced the risk of developing a recurrence.[28] Furthermore, the impact of concurrent cytology findings could not be evaluated in this study since systematic cytology analyses were not available across the four trials. Taking the risk of bias across studies and statistical heterogeneity into account, our results should be interpreted with caution.

Conclusion

Patients with NMIBC and a positive FISH after BCG induction have a higher risk for developing tumor recurrence. When FISH is performed three months following initial TURBT, the predictive value of FISH is higher compared to the FISH immediately at the end of the induction course. Pre-BCG, FISH lacks a predictive value for predicting recurrence. FISH could assist urologists in risk stratification and counseling patients prone to recur after BCG therapy, preferably within clinical trials.

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Conflict of interest

EL declares no conflicts of interests.
JO declares no conflicts of interests.
RV declares no conflicts of interests.
RL declares no conflicts of interests.
AK declares no conflicts of interests.
CD declares no conflicts of interests.
LM declares no conflicts of interests.
AA declares no conflicts of interests.
LI declares no conflicts of interests.
SS declares no conflicts of interests.
GT declares no conflicts of interests.
LB declares no conflicts of interests.
RJS declares no conflicts of interests.
TdR declares no conflicts of interests.

Author contribution

Conception and design	EL/TdR
Acquisition of data	EL/RL/AK/CD/LM/AA/LI/SS/GT/LB/TdR
Analysis and interpretation of data	EL/JO/RV/RJS/TdR
Drafting of the manuscript	EL/RV
Critical revision manuscript for important intellectual content	JO/RJS/TdR
Statistical analysis	EL/RV
Obtaining funding	TdR
Administrative, technical, or material support	RV/RL/LM/SS
Supervision	TdR

References

1. Chang SS, Boorjian SA, Chou R, et al: Diagnosis and Treatment of Non-Muscle Invasive Bladder Cancer: AUA/SUO Guideline. J. Urol. 2016; 196: 1021–1029.
2. Flaig TW, Spiess PE, Agarwal N, et al: NCCN Clinial Practice Guidelines in Oncology: Bladder Cancer. NCCN Clin. Pract. Guidel. Oncol. 2018: 1–103. Available at: https://www.nccn.org/professionals/physician_gls/pdf/bladder.pdf.
3. Bagnall PC, Catto J, Chandra A, et al: Bladder cancer: diagnosis and management. 2015: 1–57. Available at: https://www.nice.org.uk/guidance/ng2.
4. Babjuk M, Burger M, Compérat EM, et al: European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) - 2019 Update. Eur. Urol. 2019: 1–19.
5. Brausi M, Oddens J, Sylvester R, et al: Side effects of Bacillus Calmette-Guérin (BCG) in the treatment of intermediate- and high-risk Ta, T1 papillary carcinoma of the bladder: Results of the EORTC genito-urinary cancers group randomised phase 3 study comparing one-third dose with full dose an. Eur. Urol. 2014; 65: 69–76.
6. Van der Meijden AP, Sylvester RJ, Oosterlinck W, et al: Maintenance Bacillus Calmette-Guerin for Ta T1 bladder tumors is not associated with increased toxicity: Results from a European organisation for research and treatment of cancer genito-urinary group phase III trial. Eur. Urol. 2003; 44: 429–434.

7. Pansadoro V, Emiliozzi P, Paula F de, et al: Long-term follow-up of G3T1 transitional cell carcnoma of the bladder treated with intravesical bacille calmette-guérin: 18-year experience. Urology 2002; 59: 227–231.

8. Böhle A, Jocham D and Bock P: Intravesical bacillus Calmette-Guerin versus mitomycin C for superficial bladder cancer: a formal meta-analysis of comparative studies on recurrence and toxicity. J. Urol. 2003; 169: 90–95.

9. Sylvester RJ, van der Meijden AP, Witjes JA, et al: Bacillus Calmette-Guerin Versus Chemotherapy for the Intravesical Treatment of Patients With Carcinoma in Situ of the Bladder: a Meta-Analysis of the Published Results of Randomized Clinical Trials. J. Urol. 2005; 174: 86–91.

10. Mack D and Frick J: Diagnostic problems of urine cytology on initial follow-up after intravesical immunotherapy with Calmette-Guérin bacillus for superficial bladder cancer. Urol. Int. 1994; 52: 204–207.

11. Gupta M, Milbar N, Tema G, et al: Impact of intravesical therapy for non-muscle invasive bladder cancer on the accuracy of urine cytology. World J. Urol. 2019.

12. Halling KC, King W, Sokolova IA, et al: A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. J Urol 2000; 164: 1768–1775.

13. Pycha A, Mian C, Hofbauer J, et al: Does topical instillation therapy influence chromosomal aberrations in superficial bladder cancer? J. Urol. 1998; 159: 265–269.

14. Stewart LA, Clarke M, Rovers M, et al: Preferred reporting items for a systematic review and meta-analysis of individual participant data: The PRISMA-IPD statement. JAMA - J. Am. Med. Assoc. 2015; 313: 1657–1665.

15. Hayden JA, Windt DA van der, Cartwright JL, et al: Assessing Bias in Studies of Prognostic Factors. Ann. Intern. Med 2013; 158: 280–286.

16. Kipp BR, Karnes RJ, Brankley SM, et al: Monitoring intravesical therapy for superficial bladder cancer using fluorescence in situ hybridization. J. Urol. 2005; 173: 401–404.

17. Mengual L, Marín-Aguilera M, Ribal MJ, et al: Clinical Utility of Fluorescent in situ Hybridization for the Surveillance of Bladder Cancer Patients Treated with Bacillus Calmette-Guérin Therapy. Eur. Urol. 2007; 52: 752–759.

18. Whitson J, Berry A, Carroll P, et al: A multicolour fluorescence in situ hybridization test predicts recurrence in patients with high-risk superficial bladder tumours undergoing intravesical therapy. BJU Int. 2009; 104: 336–339.

19. Savic S, Zlobec I, Thalmann GN, et al: The prognostic value of cytology and fluorescence in situ hybridization in the follow-up of nonmuscle-invasive bladder cancer after intravesical Bacillus Calmette-Guérin therapy. Int. J. Cancer 2009; 124: 2899–2904.

20. Kamat AM, Dickstein RJ, Messetti F, et al: Use of fluorescence in situ hybridization to predict response to Bacillus Calmette-Guerin therapy for bladder cancer: results of a prospective trial. J. Urol. 2012; 187: 862–867.

21. Liem EI, Baard J, Cauberg EC, et al: Fluorescence in situ hybridization as prognostic

predictor of tumor recurrence during treatment with Bacillus Calmette–Guérin therapy for intermediate- and high-risk non-muscle-invasive bladder cancer. Med. Oncol. 2017; 34.

22. Lotan Y, Inman B, Davis L, et al: Evaluation of the UroVysion test to predict recurrence and/or progression of disease after BCG for primary high grade non-muscle invasive bladder cancer: results from a prospective multicenter trial. J Urol 2019.

23. Sylvester RJ, Brausi MA, Kirkels WJ, et al: Long-Term Efficacy Results of EORTC Genito-Urinary Group Randomized Phase 3 Study 30911 Comparing Intravesical Instillations of Epirubicin, Bacillus Calmette-Guérin, and Bacillus Calmette-Guérin plus Isoniazid in Patients with Intermediate- and High-Risk. Eur. Urol. 2010; 57: 766–773.

24. Holmäng S and Ströck V: Should follow-up cystoscopy in bacillus calmette-guérin-treated patients continue after five tumour-free years? Eur. Urol. 2012; 61: 503–507.

25. Kamat AM, Briggman J, Urbauer DL, et al: Cytokine panel for response to intravesical therapy (CyPRIT): nomogram of changes in urinary cytokine levels predicts patients response to Bacillus Calmette-Guérin. Eur. Urol. 2016; 15: 197–200.

26. Colombo R, Salonia A, Leib Z, et al: Long-term outcomes of a randomized controlled trial comparing thermochemotherapy with mitomycin-C alone as adjuvant treatment for non-muscle-invasive bladder cancer (NMIBC). BJU Int. 2011; 107: 912–918.

27. Arends TJ, Nativ O, Maffezzini M, et al: Results of a Randomised Controlled Trial Comparing Intravesical Chemohyperthermia with Mitomycin C Versus Bacillus Calmette-Guérin for Adjuvant Treatment of Patients with Intermediate- and High-risk Non-Muscle-invasive Bladder Cancer. Eur. Urol. 2016; 69: 1046–1052.

28. Oddens J, Brausi M, Sylvester R, et al: Final results of an EORTC-GU cancers group randomized study of maintenance bacillus calmette-guérin in intermediate- and high-risk Ta, T1 papillary carcinoma of the urinary bladder: One-third dose versus full dose and 1 year versus 3 years of maintenance. Eur. Urol. 2013; 63: 462–472.

Table 1 Risk of bias according to the Quality in Prognosis Studies (QUIPS) tool

	Mengual et al.[21]	**Savic et al.** [23]	**Kamat et al.** [24]	**Liem et al.** [25]
1. Study participation				
1) Adequate participation in the study by eligible persons	Yes	Partial	Yes	Partial
2) Description of the source population or population of interest	Partial	Yes	Yes	Yes
3) Description of the baseline study sample	Yes	Yes	Yes	Yes
4) Adequate description of the sampling frame and recruitment	Yes	Yes	Yes	Yes
5) Adequate description of the period and place of recruitment	Yes	Yes	Yes	Yes
6) Adequate description of inclusion and exclusion criteria	Partial	No	Yes	Yes
The study sample adequately represents the population of interest	*Low risk of bias*	*Moderate risk of bias*	*Low risk of bias*	*Low risk of bias*
2. Study attrition				
1) Adequate response rate for study participants	Yes	Unsure	Yes	Unsure
2) Description of attempts to collect	No	No	No	Partial
3) Reasons for loss to follow-up are provided	No	No	No	Partial
4) Adequate description of participants lost to follow-up	No	No	No	Partial
5) There are no important differences between participants who completed the study and those who did not	No	No	No	Partial
The study data available adequately represent the study	*High risk of bias*	*High risk of bias*	*High risk of bias*	*High risk of bias*
3. Prognostic factor measurement				
1) A clear definition or description of the prognostic factor is provided	Yes	Yes	Yes	Yes
2) Method of prognostic factor measurement is adequately valid and reliable	Yes	Yes	Yes	Yes
3) Continuous variables are reported or appropriate or appropriate cut points are used	Yes	Yes	Yes	Yes
4) A clear definition of the outcome is provided	Yes	Yes	Yes	Yes
5) Method of outcome measurement used is adequately valid and reliable	Yes	Yes	Yes	Yes
6) Appropriate methods of imputation are used for missing prognostic factor data	Yes	Yes	Yes	Yes
The prognostic factor is measured in a similar way for all participants	*Low risk of bias*	*Low risk of bias*	*Low risk of bias*	*Low risk of bias*
4. Outcome measurement				
1) A clear definition of the outcome is provided	Yes	Yes	Yes	Yes
2) Method of outcome measurement used is adequately valid and reliable	Yes	Yes	Yes	Yes
3) The method and setting of outcome measurement is the same for all study participants	Yes	Yes	Yes	Yes
The outcome of interest is measured in a similar way for all participants	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias
5. Study confounding				
1) All important confounders are measured	No	No	Partial	Yes
2) Clear definitions of the important confounders measured are provided	No	No	Partial	Yes

3) Measurement of all important confounders is adequately valid and reliable	No	No	Partial	Yes
4) The method and setting of confounding measurement are the same for all study participants	No	No	Yes	Partial
5) Appropriate methods are used if imputation is used for missing confounder data	NA	NA	NA	NA
6) Important potential confounders are accounted for in the study design	No	No	Yes	No
7) Important potential confounders are accounted for in the analysis	No	No	Partial	Yes
Important potential confounding factors are appropriately accounted for	*High risk of bias*	*High risk of bias*	*Moderate risk of bias*	*Low risk of bias*
6. Statistical analysis and reporting				
1) Sufficient presentation of data to assess the adequacy of the analytic strategy	Yes	Yes	Yes	Yes
2) Strategy for model building is appropriate and is based on a conceptual framework or model	Yes	Yes	Yes	Yes
3) The selected statistical model is adequate for the design of the study	Yes	Yes	Yes	Yes
4) There is no selective reporting of results	Yes	Yes	Yes	Yes
The statistical analysis is appropriate, and all primary outcomes were reported	*Low risk of bias*	*Low risk of bias*	*Low risk of bias*	*Low risk of bias*
NA = not applicable				

Table 2 Characteristics of the included clinical trials.

	Setting	N	Dates	FISH	Definition positive FISH	Definition recurrence
Mengual et al.[21]	Single center, Spain	65	Sept 2003 – Oct 2004	•Pre-BCG •Post-BCG, 3m	100 cells scored, and one of the following criteria: • ≥5 cells aneuploidy of 2 or more chromosomes (chr. 3, 7 17) • ≥20 cells with a total loss of 9p21	Histological proven bladder cancer
Savic et al.[23]	7 Swiss centers	68	Feb 2003 – Feb 2006	•Pre-BCG •Post-BCG, 3m	25 cells scored, and one of the following criteria: • ≥4 cells aneuploidy of 2 or more chromosomes (chr. 3, 7 17) • ≥12 cells with a total loss of 9p21	G3 cytology or histological proven bladder cancer
Kamat et al.[24]	Single center, USA	126	June 2005 – Feb 2012	•Pre-BCG •Post-BCG, 6w •Post-BCG, 3m •Post-BCG, 6m	25 cells scored, and one of the following criteria: • ≥4 cells aneuploidy of 2 or more chromosomes (chr. 3, 7 17) • ≥12 cells with a total loss of 9p21	Histological proven bladder cancer
Liem et al.[25]	5 Dutch centers	114	Dec 2007 – Jan 2013	•Pre-BCG •Post-BCG, 6w •Post-BCG, 3m	25 cells scored, and one of the following criteria: • ≥4 cells aneuploidy of 2 or more chromosomes (chr. 3, 7 17) • ≥12 cells with a total loss of 9p21	Histological proven bladder cancer

Table 3 Baseline patient and tumour characteristics.

	Total N=408	**Mengual et al.**[21] N=65	**Savic et al.**[23] N=68	**Kamat et al.**[24] N=142	**Liem et al.**[25] N=133
Age (years), med [IQR]	70 [62-77]	72 [64-78]	73 [63.5-79.5]	67 [58-74]	71 [64-78]
Gender, *n (%)*					
Male	324 (79.4)	57 (87.7)	60 (88.2)	107 (75.4)	100 (75.2)
Female	84 (20.6)	8 (12.3)	8 (11.8)	35 (24.6)	33 (24.8)
Follow-up (months), med [IQR]	18.8 [10.2-28.0]	14.1 [10.9-18.0]	17.9 [12.6-23.1]	26.4 [8.7-53.0]	23.3 [7.1-26.8]
History of bladder cancer, *n (%)*					
No	194 (47.5)	40 (61.5)	35 (51.5)	24 (16.9)	95 (71.4)
Yes	210 (51.5)	25 (38.5)	30 (44.1)	118 (83.1)	37 (27.8)
Missing	4 (1.0)	0	3 (4.4)	0	1 (0.8)
Prior BCG therapy, *n (%)*					
No	321 (78.7)	58 (89.2)	13 (19.1)	130 (91.6)	120 (90.2)
Yes	77 (18.9)	7 (10.8)	51 (75.0)	12 (8.4)	7 (5.3)
Missing	10 (2.4)	0	4 (5.9)	0	6 (4.5)
Stage, *n (%)*					
CIS only	72 (17.6)	11 (16.9)	31 (45.6)	7 (4.9)	23 (17.3)
Ta	159 (39.0)	21 (32.3)	21 (30.9)	67 (47.2)	50 (37.6)
T1	166 (40.7)	22 (33.8)	16 (23.5)	68 (47.9)	60 (45.1)
Missing	11 (2.7)	11 (17.0)	0	0	0
Grade, *n (%)*					
CIS only	72 (17.7)	11 (16.9)	31 (45.6)	7 (4.9)	23 (17.3)
G1	12 (2.9)	4 (6.2)	0	2 (1.4)	6 (4.5)
G2	75 (18.4)	16 (24.6)	10 (14.7)	33 (23.3)	16 (12.0)
G3	247 (60.5)	32 (49.2)	27 (39.7)	100 (70.4)	88 (66.2)
Missing	2 (0.5)	2 (3.1)	0	0	0

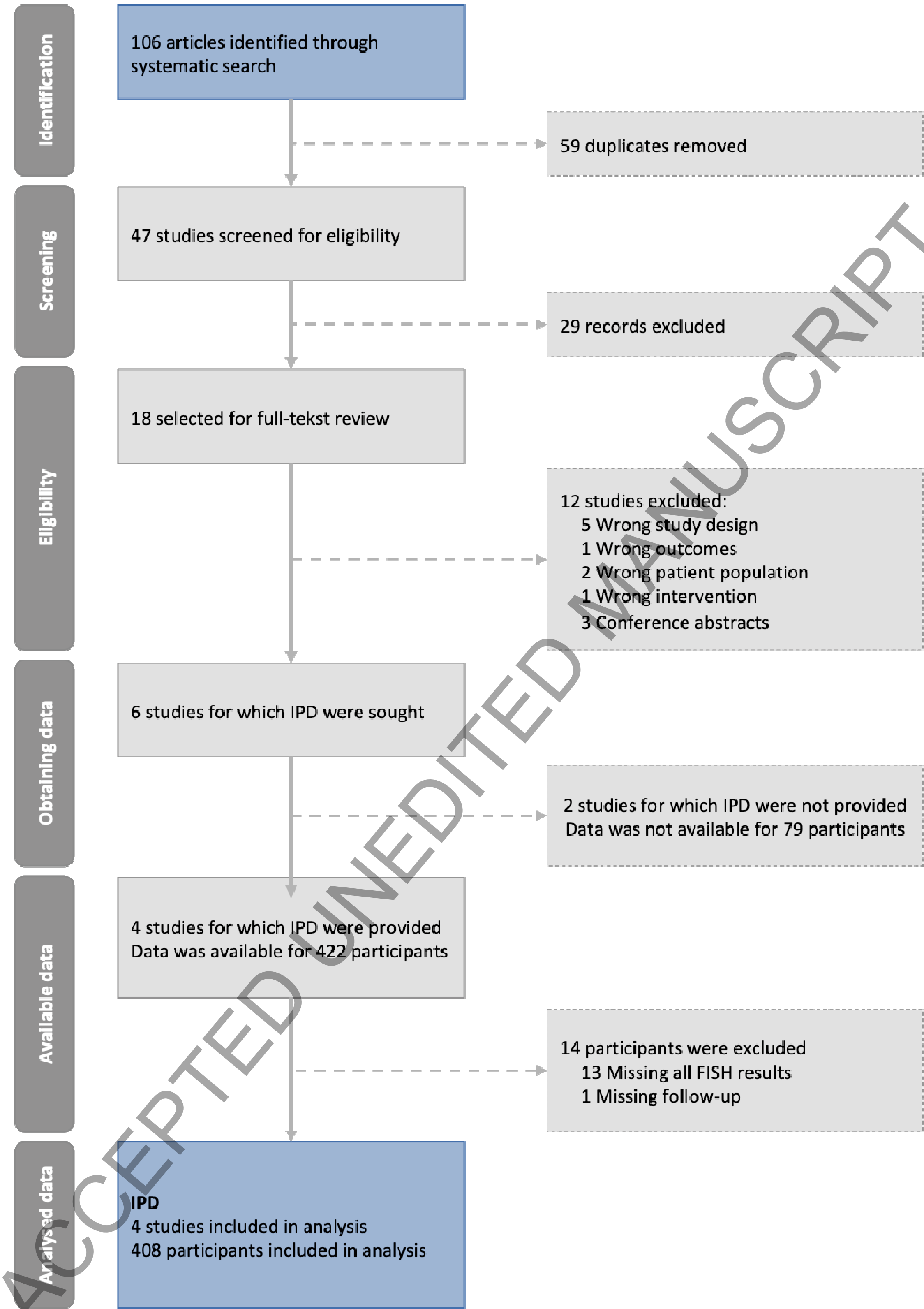
CIS, *n (%)*					
No	236 (57.8)	43 (66.2)	26 (38.2)	76 (53.5)	91 (68.4)
Yes	161 (39.5)	11 (16.9)	42 (61.8)	66 (46.5)	42 (31.6)
Missing	11 (2.7)	11 (16.9)	0	0	0
Risk group, *n (%)*					
Low-/intermediate-risk	53 (13.0)	10 (15.4)	8 (11.8)	23 (16.2)	12 (9.0)
High-risk	344 (84.3)	44 (67.7)	60 (88.2)	119 (83.8)	121 (91.0)
Missing	11 (2.7)	11 (16.9)	0	0	0

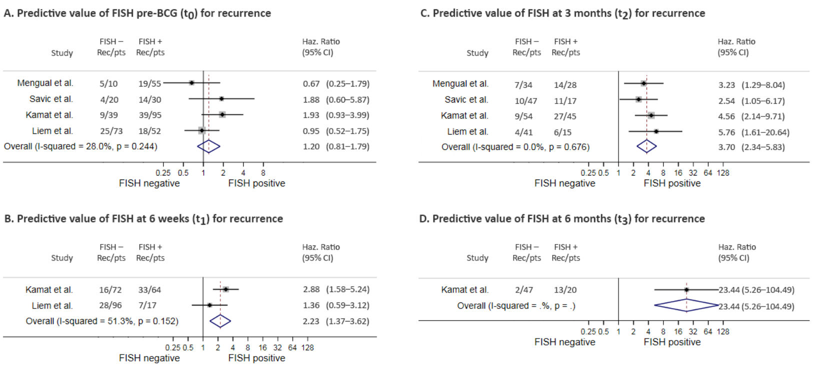
Table 4 Overview of number of recurrences and available FISH evaluations at different time points and their FISH result.

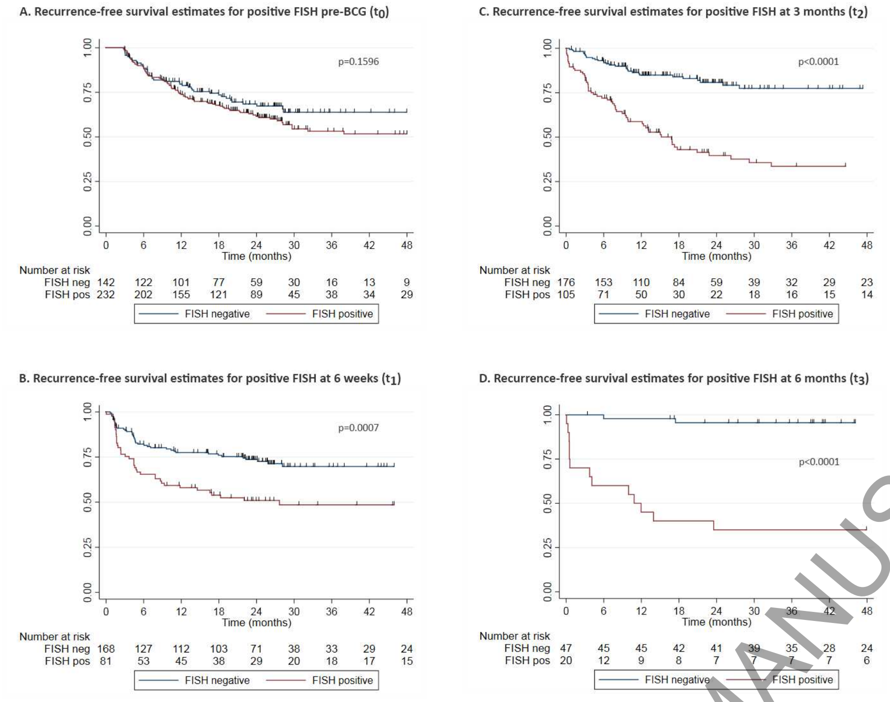
	Pre-BCG (t_0)		Post-BCG, 6 weeks (t_1)		Post-BCG, 3 months (t_2)		Post-BCG, 6 months (t_3)	
	FISH -	*FISH +*	*FISH -*	*FISH +*	*FISH -*	*FISH +*	*FISH -*	*FISH +*
Mengual et al.[21]	5/10	19/55			9/36	15/29		
Savic et al.[23]	4/20	14/30			10/48	12/20		
Kamat et al.[24]	9/39	39/95	16/72	33/64	9/54	31/49	4/49	15/22
Liem et al.[25]	25/73	18/52	28/96	7/17	7/48	10/19		
Total	43/142	90/232	44/168	40/81	35/186	68/117	4/49	15/22

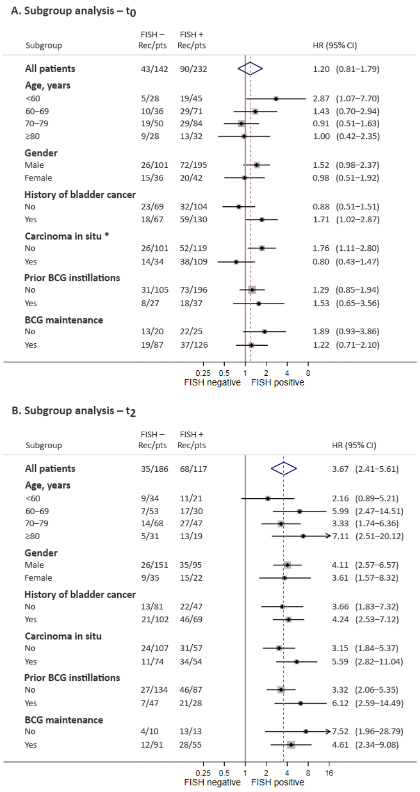
Table 5 Overview of FISH results and occurred conversions.

	Patients	Recurrence
All FISH negative	127	28
All FISH positive	99	57
FISH negative → FISH positive	34	19
Conversion between $t_0 - t_1$	*12*	*8*
Conversion between $t_0 - t_2$	*9*	*6*
Conversion between $t_1 - t_2$	*12*	*5*
Conversion between $t_2 - t_3$	*1*	*0*
FISH positive → FISH negative	126	29
Conversion between $t_0 - t_1$	*58*	*14*
Conversion between $t_0 - t_2$	*49*	*14*
Conversion between $t_1 - t_2$	*14*	*1*
Conversion between $t_2 - t_3$	*5*	*0*
Alternating FISH	22	8
FISH negative → positive → negative		
- | + | - |	*1*	*0*
- | + | - | -	*2*	*0*
| - | + | -	*1*	*0*
- | - | + | -	*2*	*0*
FISH positive → negative → positive		
+ | - | + |	*5*	*4*
+ | - | + | +	*3*	*1*
+ | + | - | +	*2*	*1*
FISH positive → negative → positive → negative	*6*	*2*


Identification
106 articles identified through systematic search
59 duplicates removed
Screening
47 studies screened for eligibility
29 records excluded
Eligibility
18 selected for full-tekst review
12 studies excluded:
5 Wrong study design
1 Wrong outcomes
2 Wrong patient population
1 Wrong intervention
3 Conference abstracts
Obtaining data
6 studies for which IPD were sought
2 studies for which IPD were not provided
Data was not available for 79 participants
Available data
4 studies for which IPD were provided
Data was available for 422 participants
14 participants were excluded
13 Missing all FISH results
1 Missing follow-up
Analysed data
IPD
4 studies included in analysis
408 participants included in analysis


A. Predictive value of FISH pre-BCG (t_0) for recurrence
Study
FISH – Rec/pts
FISH + Rec/pts
Haz. Ratio (95% CI)
Mengual et al. 5/10 19/55 0.67 (0.25–1.79)
Savic et al. 4/20 14/30 1.88 (0.60–5.87)
Kamat et al. 9/39 39/95 1.93 (0.93–3.99)
Liem et al. 25/73 18/52 0.95 (0.52–1.75)
Overall (I-squared = 28.0%, p = 0.244) 1.20 (0.81–1.79)
0.25 0.5 1 2 4 8
FISH negative
FISH positive
B. Predictive value of FISH at 6 weeks (t_1) for recurrence
Study
FISH – Rec/pts
FISH + Rec/pts
Haz. Ratio (95% CI)
Kamat et al. 16/72 33/64 2.88 (1.58–5.24)
Liem et al. 28/96 7/17 1.36 (0.59–3.12)
Overall (I-squared = 51.3%, p = 0.152) 2.23 (1.37–3.62)
0.25 0.5 1 2 4 8 16 32 64 128
FISH negative
FISH positive
C. Predictive value of FISH at 3 months (t_2) for recurrence
Study
FISH – Rec/pts
FISH + Rec/pts
Haz. Ratio (95% CI)
Mengual et al. 7/34 14/28 3.23 (1.29–8.04)
Savic et al. 10/47 11/17 2.54 (1.05–6.17)
Kamat et al. 9/54 27/45 4.56 (2.14–9.71)
Liem et al. 4/41 6/15 5.76 (1.61–20.64)
Overall (I-squared = 0.0%, p = 0.676) 3.70 (2.34–5.83)
0.25 0.5 1 2 4 8 16 32 64 128
FISH negative
FISH positive
D. Predictive value of FISH at 6 months (t_3) for recurrence
Study
FISH – Rec/pts
FISH + Rec/pts
Haz. Ratio (95% CI)
Kamat et al. 2/47 13/20 23.44 (5.26–104.49)
Overall (I-squared = .%, p = .) 23.44 (5.26–104.49)
0.25 0.5 1 2 4 8 16 32 64 128
FISH negative
FISH positive


A. Recurrence-free survival estimates for positive FISH pre-BCG (t_0)
p=0.1596
Time (months)
Number at risk
FISH neg 142 122 101 77 59 30 16 13 9
FISH pos 232 202 155 121 89 45 38 34 29
FISH negative
FISH positive
C. Recurrence-free survival estimates for positive FISH at 3 months (t_2)
p<0.0001
Time (months)
Number at risk
FISH neg 176 153 110 84 59 39 32 29 23
FISH pos 105 71 50 30 22 18 16 15 14
FISH negative
FISH positive
B. Recurrence-free survival estimates for positive FISH at 6 weeks (t_1)
p=0.0007
Time (months)
Number at risk
FISH neg 168 127 112 103 71 38 33 29 24
FISH pos 81 53 45 38 29 20 18 17 15
FISH negative
FISH positive
D. Recurrence-free survival estimates for positive FISH at 6 months (t_3)
p<0.0001
Time (months)
Number at risk
FISH neg 47 45 45 42 41 39 35 28 24
FISH pos 20 12 9 8 7 7 7 7 6
FISH negative
FISH positive



A. Subgroup analysis – t_0
Subgroup
FISH – Rec/pts
FISH + Rec/pts
HR (95% CI)
All patients 43/142 90/232 1.20 (0.81–1.79)
Age, years
<60 5/28 19/45 2.87 (1.07–7.70)
60–69 10/36 29/71 1.43 (0.70–2.94)
70–79 19/50 29/84 0.91 (0.51–1.63)
≥80 9/28 13/32 1.00 (0.42–2.35)
Gender
Male 26/101 72/195 1.52 (0.98–2.37)
Female 15/36 20/42 0.98 (0.51–1.92)
History of bladder cancer
No 23/69 32/104 0.88 (0.51–1.51)
Yes 18/67 59/130 1.71 (1.02–2.87)
Carcinoma in situ *
No 26/101 52/119 1.76 (1.11–2.80)
Yes 14/34 38/109 0.80 (0.43–1.47)
Prior BCG instillations
No 31/105 73/196 1.29 (0.85–1.94)
Yes 8/27 18/37 1.53 (0.65–3.56)
BCG maintenance
No 13/20 22/25 1.89 (0.93–3.86)
Yes 19/87 37/126 1.22 (0.71–2.10)
0.25 0.5 1 2 4 8
FISH negative FISH positive
B. Subgroup analysis – t_2
Subgroup
FISH – Rec/pts
FISH + Rec/pts
HR (95% CI)
All patients 35/186 68/117 3.67 (2.41–5.61)
Age, years
<60 9/34 11/21 2.16 (0.89–5.21)
60–69 7/53 17/30 5.99 (2.47–14.51)
70–79 14/68 27/47 3.33 (1.74–6.36)
≥80 5/31 13/19 7.11 (2.51–20.12)
Gender
Male 26/151 35/95 4.11 (2.57–6.57)
Female 9/35 15/22 3.61 (1.57–8.32)
History of bladder cancer
No 13/81 22/47 3.66 (1.83–7.32)
Yes 21/102 46/69 4.24 (2.53–7.12)
Carcinoma in situ
No 24/107 31/57 3.15 (1.84–5.37)
Yes 11/74 34/54 5.59 (2.82–11.04)
Prior BCG instillations
No 27/134 46/87 3.32 (2.06–5.35)
Yes 7/47 21/28 6.12 (2.59–14.49)
BCG maintenance
No 4/10 13/13 7.52 (1.96–28.79)
Yes 12/91 28/55 4.61 (2.34–9.08)
0.25 0.5 1 2 4 8 16
FISH negative FISH positive